PEGGY GREB (K8851-1)

Chemist Karl Kramer (left) examines the molecular interactions between biotin and avidin, a biopesticide protein in the corn. Meanwhile, technician Thomas Morgan examines insects feeding on avidin corn.

Avidin

An Egg-citing Insecticidal Protein in Corn

"Smart" is the operative word for plants and seeds that can fight off insect pests—even when stored in grain bins for months.

Among the newest crop of "smarter" plants are genetically modified corn plants containing a protein found in egg whites. Agricultural Research Service chemist Karl J. Kramer and colleagues at the Grain Marketing and Production Research Center in Manhattan, Kansas, have shown that the protein, called avidin, is a powerful growth inhibitor of insects. Their studies are now being used by a Texas-based biotechnology company to help find uses for corn containing avidin.

Such plants may become an alternative to methyl bromide—the closely regulated pesticide scheduled for phaseout in 2005.

In 1994, the U.S. Department of Agriculture launched a national integrated pest management (IPM) initiative in which the ARS role has been to develop biologically based alternatives for controlling insect pests, weeds, and crop diseases. Plant resistance and biological control are key parts of the IPM program.

Each year, stored-grain insects cause multimillion-dollar losses for U.S. producers of stored commodities such as corn, wheat, rice, and grain sorghum.

Biotin—a common vitamin—is essential for insect growth and development. Avidin restricts the availability of biotin, so the insect stops developing and dies. Avidin may have a similar role in chicken egg whites—to protect chicken embryos from disease-causing organisms that require biotin to grow.

"As a biopesticide, avidin is better than *Bacillus thuringiensis* (Bt) in corn because it has a knockout punch that hits a broader range of insects," says Kramer. Insertion of the genes of Bt, a bacterial biocontrol agent, allows corn plants to resist primarily moths and only a few species of beetles.

Biopesticide Tests

Kramer and his collaborators first evaluated avidin as a biopesticide in the early 1990s. He, ARS chemist Brenda Oppert, ARS biological research technician Thomas D. Morgan, and Thomas Czapla, an entomologist at Pioneer Hi-Bred International in Johnston, Iowa, studied the protein in artificial insect diets. More recently, they studied transgenic corn (corn in which the genes were modified) that contained avidin—supplied by ProdiGene of College Station, Texas.

When kernels of avidin corn were infested with Angoumois grain moths or maize weevils, most of the larvae died inside kernels that contained at least 20 parts per million of avidin. Cornmeal obtained from the avidin corn was resistant to all common U.S. storage pests. Meal with more than 100 ppm avidin killed larvae of lesser grain borers, red flour beetles, confused flour beetles, sawtoothed grain beetles, flat grain beetles, warehouse beetles, Indianmeal moths, and Mediterranean flour moths.

While avidin was lethal to many stored-grain pests, one species escaped death in the ARS studies. *Prostephanus truncatus,* the larger grain borer—rarely found in the United States—was not affected. This insect's tolerance could be from bacteria living within its cells that may synthesize biotin or from an enzyme in its gut that may inactivate avidin.

Overall, effectiveness of avidin corn hinged on the amount eaten and how long it was consumed. Most of the insects fed corn with 100 ppm avidin were unable to grow and develop, and about half were killed by concentrations as low as 30 ppm.

"Avidin corn can benefit farmers by offering grain buyers a higher quality corn that can be stored longer without pesticides," says Kramer. "It also means that products made from avidin corn will have a longer shelf life. Because the corn has this property, one proposed use of it is as a background germplasm for production of other valuable recombinant proteins in corn."

Avidin corn is now grown by U.S. farmers under contract with Stauffer Biotech in Aurora, Nebraska. The avidin compound is currently used in medical and biochemical kits as a diagnostic protein. When produced from chicken eggs, avidin sells for about $3,000 per gram. But the cost is lower when the chemical is produced from corn—now being done by the Sigma-Aldrich chemical company in St. Louis, Missouri.

Safety

Although avidin is already a common protein in human diets, the Food and Drug Administration will require a thorough risk assessment of avidin corn before approving it as a food or feed product.

One challenge researchers encountered was determining the concentration of avidin in corn kernels. Only half of the kernels in the corn varieties contained avidin because male sterility occurs in avidin-positive plants. An immunoassay can measure avidin concentration, but this method requires extracting the protein from ground-up kernels.

Kramer asked ARS agricultural engineer Floyd E. Dowell and entomologist James E. Throne to use less destructive near-infrared technology to analyze kernels. This helped solve the problem of distinguishing kernels with high avidin content.

"We want to do more work to ensure that every kernel has a consistent amount of avidin," says Kramer.

The only insects affected are those that actually feed on plants because, in this case, the plant is the sole source of the biopesticide. That means beneficial insects and others that don't feed on the crop would not be affected. Groundwater, too, is protected from contamination because avidin is a biodegradable protein.

PEGGY GREB (K8854-1)

Maize weevil, *Sitophilus zeamaise.*

Avidin imparts to corn a kind of host plant resistance that can last from the field to the storage bin. Unlike chemical insecticidal sprays that can be washed off by rain or inactivated by ultraviolet rays, avidin works regardless of the weather.

PEGGY GREB (K8853-1)

Technician Thomas Morgan aspirates maize weevils into a glass vial. Adult females will be placed on kernels of avidin maize to test the kernel's resistance to the pest.

To avoid any problems that could result from avidin being expressed in corn pollen, Kramer says that molecular techniques will use specific promoters that place avidin not in the pollen—but in the seed, roots, stems, or leaves.—By **Linda McGraw,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/cppvs.htm.

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